### BACKGROUND

Ziv-AFLibercept or ziv-AFL (Zaltrap®) is a Fc-fusion protein used in the treatment of colorectal cancer. Changes in the structure or aggregation, which may arise from handling and storage, may affect the efficacy of the treatment and it could cause severe immune reactions to patients. The shelf life indicated by the manufacturer for the unopened vial is 3 years; nothing is declared about the surplus of opened vials.

### OBJECTIVE

To compare the biophysical stability of ziv-AFL (zaltrap®) stored refrigerated at 4°C and at room temperature (20°C) protected from light for two weeks.

### MATERIALS AND METHODS

**Materials**: 3 independent samples of fresh ziv-AFLibercept were collected from hospital and stored in amber glass vials protected from light and stored at 4°C and room temperature (20°C).

**Aggregates**: Size Exclusion chromatography (SEC). The analysis was performed by liquid chromatography using an Agilent 1100 chromatograph equipped with a quaternary pump, degasser, autosampler, column oven and photodiode array detector (Agilent Technologies).

**Particle**: Dynamic Light Scattering (DLS) readings were carried out in a Protein Solutions DynaPro-99 System Dynamic Light Scattering Module equipped with a Temperature Control Micro Sampler (Wyatt, Santa Bárbara, California, USA) for obtaining the hydrodynamic radius and polydispersity.

**Tertiary structure**: Intrinsic Tryptophan Fluorescence measurements were carried out on a Cary Eclipse spectrofluorometer (Agilent, Santa Clara, CA, USA). Each spectrum was reduced to a single adimensional number (centroid):

\[ c = \frac{\sum_{i} f_i}{\sum_{i} i} \]

### RESULTS

#### Stability study of samples stored at 4°C

Significant changes on the monitored physicochemical parameters of the ziv-AFL samples stored at 4°C were not detected by any of the techniques applied. With regards aggregation, the population of the dimers remained constant until day 14 (Figure 1). For particle (Table 1), although there was detected slight increase in the HR of the main size distribution, the intensity scattered by high molecular weight species did not decrease. Concerning tertiary structure, this remained unaffected (Figure 2), deduced form the value of the centroid at the last checked day which was identical to that obtained at day 0.

#### Stability study of samples stored at room temperature

Differences arise in the ziv-AFL samples stored at room temperature. Regarding aggregation, a clear increase in the population of dimers was detected by SEC indicating incipient aggregation. Nonetheless, particle increase was not observed by DLS. The analysis of the tertiary structure showed a slight decrease in the fluorescence intensity which was accompanied by a slight shift in the centroid value, indicative of some kind of conformational changes.

### Table 1. Stability study results of the ziv-AFL samples stored at 4°C and at room temperature for 14 days by DLS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average hydrodynamic radius (nm) (± standard deviation)</th>
<th>Polydispersity (%) of the main population</th>
<th>% of intensity dispersion of the main population</th>
<th>% of intensity dispersion of high molecular weight entities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ziv-AFL Day 0</td>
<td>10.1 ± 0.4</td>
<td>9.1</td>
<td>87.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Ziv-AFL Day 14 (stored at 4°C)</td>
<td>11.5 ± 0.8</td>
<td>9.1</td>
<td>87.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Ziv-AFL Day 14 (stored at 4°C)</td>
<td>11.3 ± 0.6</td>
<td>9.1</td>
<td>86.7</td>
<td>11.9</td>
</tr>
<tr>
<td>Ziv-AFL Day 14 (stored at 20°C)</td>
<td>10.1 ± 0.6</td>
<td>9.1</td>
<td>88.1</td>
<td>12.2</td>
</tr>
<tr>
<td>Ziv-AFL Day 14 (stored at 20°C)</td>
<td>11.5 ± 0.8</td>
<td>9.1</td>
<td>88.1</td>
<td>12.2</td>
</tr>
</tbody>
</table>

### CONCLUSION

Ziv-AFlibercept (Zaltrap®) remained stable for 14 days regarding visual appearance, aggregates, particulate and tertiary structure when stored at 4°C. However storage at room temperature promoted modifications on ziv-AFL. This result encourages more studies with the samples stored at 4°C to establish in-use stability of Zaltrap® opened vials.